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US DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE ATTORNEY'S DOCKET NUMBER FORM PTO-1390 (REV. 11-2000) 21195 PUS TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) U.S. APPLICATION NO. (If known, see 37 CFR 1 5) 1 Gobenbury 2 () CONCERNING A FILING UNDER 35 U.S.C. 371 PRIORITY DATE CLAIMED INTERNATIONAL APPLICATION NO. INTERNATIONAL FILING DATE 27 April 1999 13 April 2000 PCT/EP00/03306 ARRANGEMENT FOR OPTICAL EVALUATION OF AN OBJECT ARRAY TITLE OF INVENTION APPLICANT(S) FOR DO/EO/US STEFAN SCHMIDT Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information: 1. X This is a FIRST submission of items concerning a filing under 35 U.S.C. 371. This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371. This is an express request to begin national examination procedures (35 U.S.C. 371 (f)). The submission must include items (5), (6), (9) and (21) indicated below. 4. X The US has been elected by the expiration of 19 months from the priority date (Article 31). 5. X A copy of the International Application as filed (35 U.S.C. 371(c)(2)) is attached hereto (required only if not communicated by the International Bureau). b. X has been communicated by the International Bureau. c. is not required, as the application was filed in the United States Receiving Office (RO/US). 6. X An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)). a. X is attached hereto. b. has been previously submitted under 35 U.S.C. 154(d)(4). 7. X Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)). a. are attached hereto (required only if not communicated by the International Bureau). b. X have been communicated by the International Bureau... have not been made; however, the time limit for making such amendments has NOT expired. have not been made and will not be made. 18. X An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)). 9. X An Unexecuted oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)). Items 11 to 20 below concern document(s) or information included: An Information Disclosure Statement under 37 CFR 1.97 and 1.98. An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included. 13. A FIRST preliminary amendment. A SECOND or SUBSEQUENT preliminary amendment. 15. X A substitute specification. A change of power of attorney and/or address letter. 17. A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825. 18. A second copy of the published international application under 35 U.S.C. 154(d)(4). A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4). Other items or information:

U.S. APPLICATION TO CAKNOWN	0'0'9'890	INTERNATIONAL APPLICATION NO PCT/EP00/03306			attorney's doc 21195.PUS	CKET NUMBER	
21. X The following fees are submitted:					CALCULATIONS PTO USE ONLY		
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International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)							
International prelimin and all claims satisfie							
ENTER APPROPRIATE BASIC FEE AMOUNT =					\$3,480.00		
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CLAIMS	NUMBER FILED	NUMBER EXTRA		RATE	\$		
Total claims	14 - 20 =	0	х	\$18.00	\$0.00		
Independent claims	1 - 3 =	0	x	\$84.00	\$0.00		
MULTIPLE DEPENI	DENT CLAIM(S) (if a		<u> </u>	+ \$280.00	\$280.00		
<u></u>	TOTA	L OF ABOVE CALCU	LA	TIONS =	\$0.00		
Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.				\$0.00			
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TOTAL NATIONAL FEE =					\$3,890.00		
Fee for recording the enclosed assignment (37 CFR 1.21 (h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property					\$0.00		
TOTAL FEES ENCLOSED =				\$3,890.00			
					Amount to be refunded:	\$	
					charged:	\$	
 a. X A check in the amount of \$3890.00 to cover the above fees is enclosed. b. Please charge my Deposit Account No. 18-0990 in the amount of \$3890.00 to cover the above fees. A duplicate copy of this sheet is enclosed. 							
c. X The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 18-0990. A duplicate copy of this sheet is enclosed.							
d. Fees are to be charged to a credit card. WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PVO-2038.							
NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status							
SEND ALL CORRESP	ONDENCE TO:			STONIA	URE:		
Eugene E. Renz, Jr.,				/ _/ _		,	
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JC13 Rec'd PCT/PTO 26 OCT 2001

ARRANGEMENT FOR OPTICAL EVALUATION OF AN OBJECT ARRAY

FIELD OF THE INVENTION

The present invention relates to improvements the arrangement for optical evaluation of an object array.

BRIEF DESCRIPTION OF THE DRAWINGS

These and other objects of the present invention and various features and details of the operation and construction thereof are hereinafter more fully set forth with reference to the accompanying drawings, wherein:

The invention is explained in greater detail on the basis of an exemplary embodiment, which is illustrated in the drawing:

Fig. 1 shows the entire beam path, for example, in fluorescence measurement;

- Fig. 2 shows the beam path in absorption measurement;
- Fig. 3 shows the beam path in luminescence measurement; and
- Fig. 4 shows the beam path without the MLA.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

The optical assembly is divided into three essential components.

- 1. A microlens array (MLA) (2) for focusing light into small areas (sample volumes) of the wells (1a-1d) of a microtiter plate (MTP) (1), which are filled with a sample substance. In the case of fluorescence or luminescence applications, the MLA (2) also serves to collect light emitted from the sample volume.
- 2. A telescope arrangement of lenses 3, 11 and of a collimator (14) for illuminating the microlens array 2. As the light source, the output of an optical fiber may be used, or, as represented in the Figures, the lamp itself.
- 3. A field lens (3) and an objective lens (6) for telecentric imaging of the pupils of the MLA (2) on a CCD-array detector (7).

The microlens array consists of a regular arrangement of small lenses or objectives. Preferably, the microlenses are arranged to form a rectangular grid. In any case, the arrangement of the microlenses is adapted to the geometry of the sample container array or of the MTP, respectively.

The front lens (3) of the telescope for illumination, which lens faces the MLA (2), also serves as field lens for the telecentric imaging of the MLA pupils.

1. The excitation beam path

The light exiting from the optical fiber (15) is collected by a collimator (14). This collimator (14), together with the small telescope lens (11), which has a short focal length, images the optical fiber output into the intermediate image plane (9) of the telescope 3, 11. The large telescope lens (3), which has a long focal length, picks up the light from the intermediate image plane (9) and transforms it into a bundle of small divergence, by which the MLA (2) is illuminated on its side facing away from the sample. Each individual lens (2a..2d) of the MLA (2) then focuses the light into a respective well (1a..1d) of the microtiter plate (1).

The aperture diaphragm (12) of the illuminating telescope and attenuating filter (13) are disposed between the collimator (14) and the small telescope lens (11). The aperture diaphragm (12) defines the shape of the beam cross-section and keeps superfluous light away from the beam path. This serves to reduce signal cross-talk and scattered light background in the detection beam path. The aperture diaphragm (12) is disposed in a plane which is conjugated to the plane of the microlens pupils. Thus, the aperture lens (12) forms a small-scale copy of the external outline of the MLA (2). The aperture diaphragm (12) may also be embodied as a disk diaphragm array for improved reduction of scattered light.

The field diaphragm (9a) of the illuminating telescope is disposed in the intermediate image plane (9). The intermediate image plane (9) is imaged into the wells (1a..1d) of the MTP (1) by the large telescope lens (3) and the lenses (2a..2d) of the MLA (2). Thus, the field diaphragm (9a) defines the bundle cross-section of the light within the wells (1a..1d).

The excitation filter (10) serves to define the spectral range of the illumination.

The light from the samples is reflected into the beam path, which is also used for detection, via the mirror (8).

Arranging said mirror 8 in front of the objective 6 facilitates the selection of a different mode of illumination.

If the device is used for fluorescence analysis, the mirror (8) is designed as a dichroic beam splitter. The spectral range of the exciting light is reflected, while the spectral range to be detected is transmitted. If the device serves to detect luminescence, reflexing by the mirror 8 can be omitted as well as the illumination, and the beam path will then only comprise the detection beam path, as set forth below.

2. Detection beam path

The light emitted from the sample volume is collimated by the microlenses (2a..2d). One microlens (2a..2d) is assigned to each well (1a..1d) of the MTP (1). The collimated light exiting from the pupils of the microlenses is focused on the intermediate image plane (4), which is conjugated to the intermediate image plane (9) on the excitation side, by the large telescope lens (3), which also serves as field lens for pupil imaging. Thus, the images of the sample volumes from all wells (1a..1d) of the MTP (1) are superimposed in the intermediate image plane (4). The diaphragm (4a) defines the observed sample volume in each well. Preferably, the diaphragm (4a) is the same size as the field diaphragm (9a) of the illumination.

The diaphragm (4a) also constitutes the aperture diaphragm for pupil imaging. The imaging of the pupils of the microlenses (2a..2d) onto the CCD-array detector (7) is carried out by the objective lens(6).

The emission filter (5) serves to define the detected spectral range.

The reflecting mirrors (16,17,18) serve to bring the beam path into a compact shape. To this end, multiple folding by a multiplicity of mirrors is conceivable.

Description of modes of measurement

In principle, different methods of measurement are applicable to the samples contained in the MTP. Based on the above-described beam path, the measurements according to a method may be carried out in several wells at the same time. So far, the following methods of measurement have been taken into consideration:

- 1. Absorption
- 2. Fluorescence
- 3. Luminescence
- 4. Time-dependent fluorescence detection
- 5. Polarization-dependent fluorescence (absorption)

Absorption

In absorption measurement, only the excitation beam path is used.

The beam splitter 8 may be replaced by a full mirror. The detection of the light transmitted through the sample is effected by means of a photodiode array (19), which is located as closely behind the sample containers as possible.

The above-described beam path allows the side of the MLA facing away from the samples to be homogeneously illuminated so that each well is traversed by light of the same intensity. For absorption purposes, the MLA is to be adapted such that the walls of the wells do not limit the beams formed by the microlenses and that each light bundle impinges fully on its associated detector surface (19a..19d) of the photodiode array.

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This means that, for certain MTPs or sample containers, exchangeable MLAs and field diaphragms 9a may be provided, which are optimized with regard to their focal lengths and radiuses of curvature.

Fluorescence

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In fluorescence measurement, excitation is effected in the same manner as in the case of absorption measurement. However, the mirror (8) is replaced by a dichroic beam splitter having high transmission for the light emitted by the sample.

The design of the MLA depends on the excitation, which should be as selective as possible, and on the detection of a small volume within the well, i.e. a sufficient chromatic correction requires a high numeric aperture (equal to, or greater than, 0.5).

Luminescence

Since the sample emits light by itself, only the detection beam path is used. The beam splitter 8 may be swivelled out.

It is possible, in general and also in absorption measurements, to omit the MLA and to image an image of the MTP bottom directly onto the CCD-array detector. In this case, the entire plate can be read at once, regardless of the number of wells it contains. Although this means slight losses in sensitivity, all channels may be read simultaneously even where there are a large number of channels.

Fluorescence detection over time

In time-dependent fluorescence detection, the same beam path is used as in fluorescence detection.

Excitation is effected by a light source capable of generating short light pulses (ca. 1 ns), i.e., for example, a suitable flash lamp.

The detector used should be capable of carrying out, after a delay of about the length of the excitation impulse, a measurement having an integration time of about the same length, synchronized to the illumination clock.

A microchannel-amplified camera is suitable for this purpose.

The intensity of fluorescence remaining after said delay is measured.

Polarization-dependent fluorescence

This requires a polarization-maintaining optical system. The fluorescence intensity in the polarization direction orthogonal to the excitation light is measured.

To this end, polarizing filters, which preferably polarize perpendicular to each other, may be provided in front of filters 5 and 10.

Even though a particular embodiment of the invention has been illustrated and described herein, it is not intended to limit the invention and changes and modifications may be made therein within the scope of the following claims.

4/prts

Translation into English

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Title:

Arrangement for optical evaluation of an object array

In the Figures:

- Fig. 1 shows the entire beam path, for example in fluorescence measurement;
 - Fig. 2 shows the beam path in absorption measurement;
 - Fig. 3 shows the beam path in luminescence measurement;
 - Fig. 4 shows the beam path without the MLA.

15 Description of the beam path

The optical assembly of [sic!] is divided into three essential components.

- A microlens array (MLA) (2) for focusing light into small areas (sample volumes) of the
 wells (1a-1d) of a microtiter plate (MTP) (1), which are filled with a sample substance. In the case of fluorescence or luminescence applications, the MLA (2) also serves to collect light emitted from the sample volume.
 - 2. A telescope arrangement of lenses 3, 11 and of a collimator (14) for illuminating the microlens array 2. As the light source, the output of an optical fiber may be used, or, as represented in the Figures, the lamp itself.
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- The microlens array consists of a regular arrangement of small lenses or objectives.

 Preferably, the the microlenses are arranged to form a rectangular grid. In any case, the arrangement of the microlenses is adapted to the geometry of the sample container array or of the MTP, respectively.
- The front lens (3) of the telescope for illumination, which lens faces the MLA (2), also serves as field lens for the telecentric imaging of the MLA pupils.

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The aperture diaphragm (12) of the illuminating telescope and attenuating filter (13) are disposed between the collimator (14) and the small telescope lens (11). The aperture diaphragm (12) defines the shape of the beam cross-section and keeps superfluous light away from the beam path. This serves to reduce signal cross-talk and scattered light background in the detection beam path. The aperture diaphragm (12) is disposed in a plane which is conjugated to the plane of the microlens pupils. Thus, the aperture lens (12) forms a smallscale copy of the external outline of the MLA (2). The aperture diaphragm (12) may also be embodied as a disk diaphragm array for improved reduction of scattered light.

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The field diaphragm (9a) of the illuminating telescope is disposed in the intermediate image plane (9). The intermediate image plane (9) is imaged into the wells (1a..1d) of the MTP (1) by the large telescope lens (3) and the lenses (2a..2d) of the MLA (2). Thus, the field diaphragm (9a) defines the bundle cross-section of the light within the wells (1a..1d).

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The excitation filter (10) serves to define the spectral range of the illumination.

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via the mirror (8).

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Arranging said mirror 8 in front of the objective 6 facilitates the selection of a different mode

of illumination.

If the device is used for fluorescence analysis, the mirror (8) is designed as a dichroic beam splitter. The spectral range of the exciting light is reflected, while the spectral range to be detected is transmitted. If the device serves to detect luminescence, reflexion by the mirror 8 can be omitted as well as the illumination, and the beam path will then only comprise the detection beam path, as set forth below.

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2. Detection beam path

The light emitted from the sample volume is collimated by the microlenses (2a..2d). One microlens (2a..2d) is assigned to each well (1a..1d) of the MTP (1). The collimated light exiting from the pupils of the microlenses is focused on the intermediate image plane (4), which is conjugated to the intermediate image plane (9) on the excitation side, by the large telescope lens (3), which also serves as field lens for pupil imaging. Thus, the images of the sample volumes from all wells (1a..1d) of the MTP (1) are superimposed in the intermediate image plane (4). The diaphragm (4a) defines the observed sample volume in each well. Preferably, the diaphragm (4a) is the same size as the field diaphragm (9a) of the illumination.

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The reflecting mirrors (16,17,18) serve to bring the beam path into a compact shape. To this end, multiple folding by a multiplicity of mirrors is conceivable.

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In principle, different methods of measurement are applicable to the samples contained in the MTP. Based on the above-described beam path, the measurements according to a method may be carried out in several wells at the same time. So far, the following methods of measurement have been taken into consideration:

- 1. Absorption
- 2. Fluorescence
- 3. Luminescence
- 4. Time-dependent fluorescence detection
- 5. Polarization-dependent fluorescence (absorption???) [sic!]

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<u>Absorption</u>

In absorption measurement, only the excitation beam path is used.

The beam splitter 8 may be replaced by a full mirror. The detection of the light transmitted through the sample is effected by means of a photodiode array (19), which is located as closely behind the sample containers as possible.

The above-described beam path allows the side of the MLA facing away from the samples to be homogeneously illuminated so that each well is traversed by light of the same intensity.

10 For absorption purposes, the MLA is to be adapted such that the walls of the wells do not limit the beams formed by the microlenses and that each light bundle impinges fully on its associated detector surface (19a..19d) of the photodiode array.

This means that, for certain MTPs or sample containers, exchangeable MLAs and field diaphragms 9a may be provided, which are optimized with regard to their focal lengths and radiuses of curvature.

Fluorescence

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In fluorescence measurement, excitation is effected in the same manner as in the case of absorption measurement. However, the mirror (8) is replaced by a dichroic beam splitter having high transmission for the light emitted by the sample.

The design of the MLA depends on the excitation, which should be as selective as possible, and on the detection of a small volume within the well, i.e. a sufficient chromatic correction requires a high numeric aperture (equal to, or greater than, 0.5).

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Fluorescence detection over time

In time-dependent fluorescence detection, the same beam path is used as in fluorescence detection.

Excitation is effected by a light source capable of generating short light pulses (ca. 1 ns), i.e., for example, a suitable flash lamp.

The detector used should be capable of carrying out, after a delay of about the length of the excitation impulse, a measurement having an integration time of about the same length, synchronized to the illumination clock.

10 A microchannel-amplified camera is suitable for this purpose.

The intensity of fluorescence remaining after said delay is measured.

Polarization-dependent fluorescence

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This requires a polarization-maintaining optical system. The fluorescence intensity in the polarization direction orthogonal to the excitation light is measured.

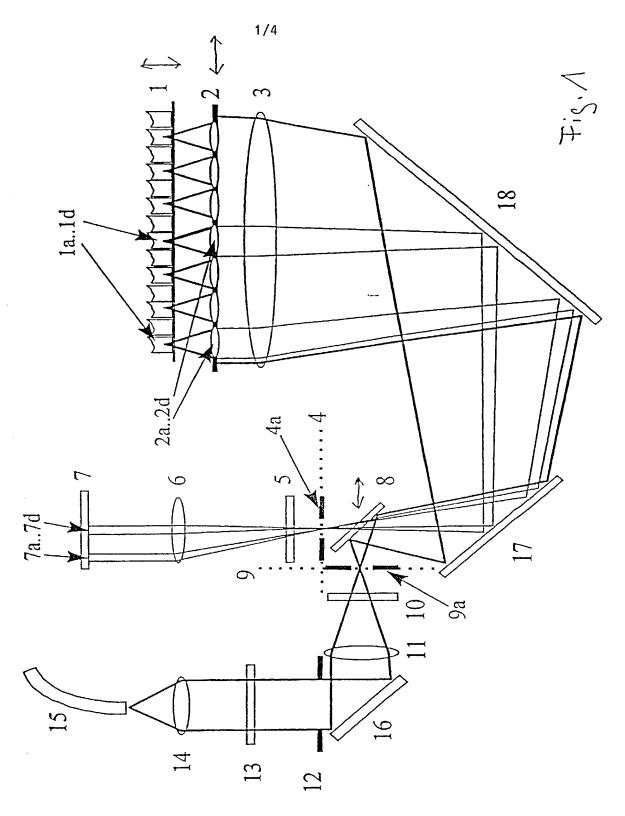
To this end, polarizing filters, which preferably polarize perpendicular to each other, may be provided in front of filters 5 and 10.



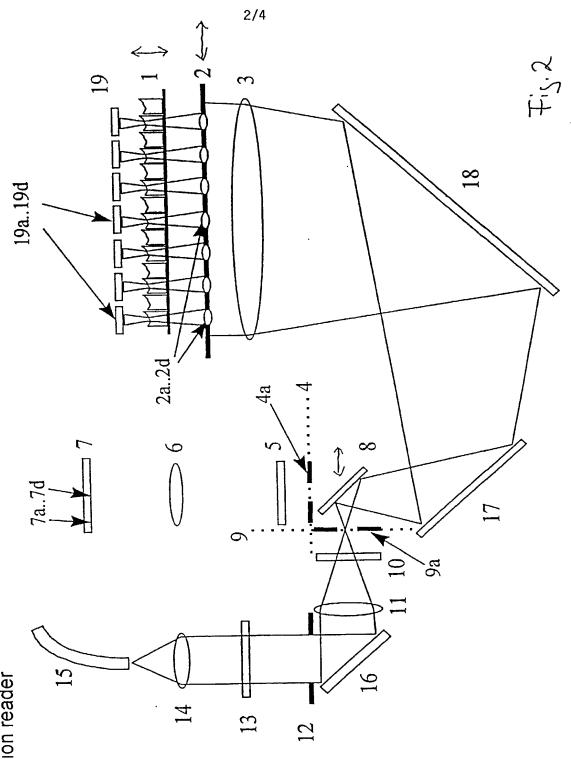
Amended claims

- 1. Arrangement for optical evaluation of an object array (1), comprising
- a detector array (7),
- a microlens array (2), which is disposed in front of the object array (1), as viewed in the direction of the detector array (7),
- a field lens (3), which is disposed in front of the object array (1), as viewed in the direction of the detector array (7),
- a light source (15), the radiation of which is coupled in by means of a beam splitter (8) between the field lens (3) and an objective (6),
- wherein the objective (6), together with the field lens (3), simultaneously images all pupils of the microlens array (2) onto the detector array (7).
- 2. An arrangement as claimed in Claim 1, wherein the field lens (3) and a further lens (11) form a telescopic arrangement which illuminates the object array (1) with light from the light source (15).
- 3. An arrangement as claimed in any of the preceding Claims, comprising a diaphragm (4a) disposed between the field lens (3) and the objective (6), wherein the beam splitter (8) is located between the diaphragm (4a) and the field lens (3).
- 4. An arrangement as claimed in any of the preceding Claims, wherein the field lens (3) and the objective (6) effect telecentric imaging of the pupil plane of the microlens array (2) onto the detector array (7).
- 5. An arrangement as claimed in any of the preceding Claims, wherein one or more reflecting elements (17, 18) for folding the beam path for illumination and/or detection are provided between the field lens (3) and the diaphragm (4a).
- 6. An arrangement as claimed in any of the preceding Claims, wherein the object array (1) is slideable, at least vertically to the axis of illumination.
- 7. An arrangement as claimed in any of the preceding Claims, wherein the light source (15) is intermittently switchable and a detection synchronized to the illumination clock, preferably a deferred detection, is possible so as to allow a time-dependent fluorescence measurement.
- 8. An arrangement as claimed in Claim 7, comprising a flash lamp as the light source (15).

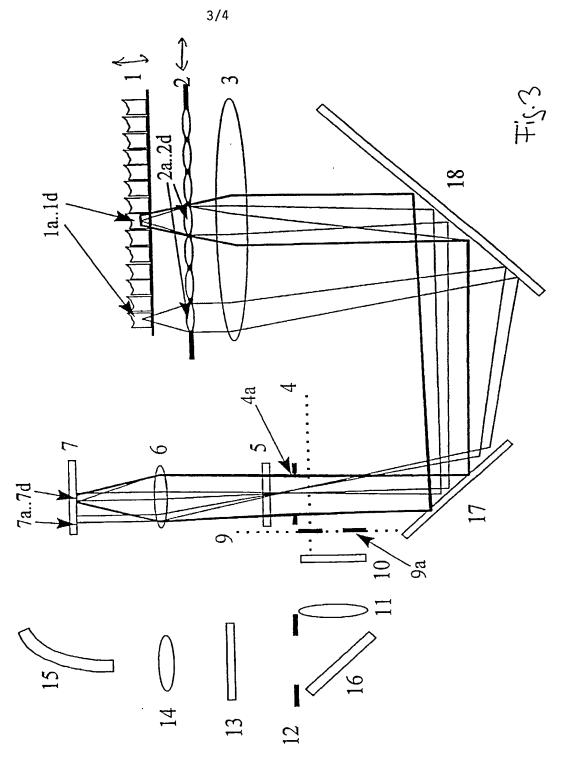
- PAR 34 MINOT
 - 9. An arrangement as claimed in any of the preceding Claims, wherein the microlens array (2) can be swivelled out of the beam path for observing the entire object array (1) and/or is exchangeable for adjustment to different measuring applications.
 - 10. An arrangement as claimed in any of the preceding Claims, wherein the light source (15) can be switched off for luminescence detection and/or a coupling element (8) for coupling in the radiation of the light source (15) can be swivelled out.
 - 11. An arrangement as claimed in any of the preceding Claims, wherein a second detector array is disposed behind the object array (1) in the illumination direction for absorption measurement.
 - 12. An arrangement as claimed in any of the preceding Claims in a combined device for measuring at least one of the following phenomena on the object array (1): fluorescence, time-dependent fluorescence, luminescence, and absorption.
 - 14. The use of an arrangement as claimed in any of the Claims 1 to 11 as a reader for microtiter plates.



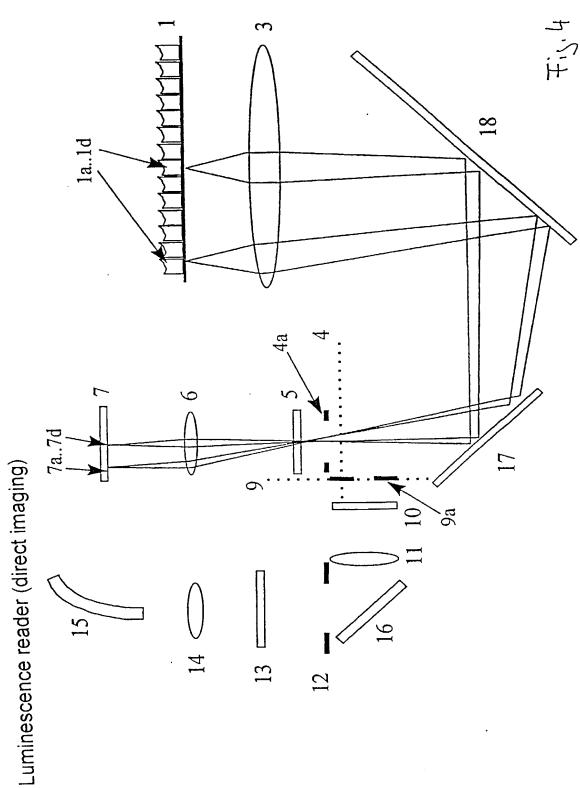
Fluorescence reader



Absorption reader



Luminescence reader



DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION ENGLISH LANGUAGE DECLARATION

As a below named inventors, we hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

We believe we are an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

ARRANGEMENT FOR OPTICAL EVALUATION OF AN OBJECT ARRAY

International Application No.: PCT/EP00/03306

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, Section 1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

PAGE 1 OF 3

ENGLISH LANGUAGE DECLARATION

PRIOR POREI	PRIORITY CLAIMED		
<u>19919092.</u> 5 (Number)	Germany (Country)	27/Apr./99 (Day/Mo/Yr Filed)	- 🗹 🗆
	306 (EP/PCT OFFIC	E) 13/Apr./00	Yes No
(Number)	(Country)	(Day/Mo/Yr Filed)	Yes No
(Number)	(Country)	(Day/Mo/Yr Filed)	Yes No
Section 120 insofar as application in the mann States Code material in Regulations of the price	of any United 8 the subject ma is not disclosed er provided by t er Section 112, aformation as deliberties 1.56(a)	tates Application (atter of each of in the prior Unite he first paragraph I acknowledge the efined in Title 3 which occurred bet and the national o	United States Code, s) listed below and, the claims of this ed States application of Title 35, United at duty to disclose 7, Code of Federal tween the filing date or PCT international
(Applicatio	n Serial No.)	Filing Date	(Status)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

(Application Serial No.) Filing Date

PAGE 2 OF 3

(Status)

THE LIBERT OF SHEETEN NOW.

following attorneys to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:
EUGENE E. RENZ, JR. / Reg. No. 19.557
Send Correspondence to: EUGENE E. RENZ, JR., P.C. P.O. Box 2056 205 North Monroe Street Media, PA 19063-9056
Direct Telephone Calls to: Eugene E. Renz, Jr. (610) 565-6090
Full name of sole or first joint inventor Stefan Schmidt
Inventor's signature
Date 30.0d.2001 Country of Citizenship Germany
Residence Buchaer Strasse 6b, D-07745 Jena, Germany
Post Office Address Buchaer Strasse 6b. D-07745 Jena. Germany
Full name of second joint inventor, if any
Inventor's signature
Date Country of Citizenship
Residence
Post Office Address
Full name of third joint inventor, if any
Inventor's signature
Date Country of Citizenship
Residence

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